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The Impact of Phenology, Exposure and Instar Susceptibility on Insecticide Effects on a Chrysomelid Beetle Population

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Abstract: Direct topical impact of an insecticide spray on a population of a non-target leaf-eating beetle, *Gastrophysa polygoni*, was studied, and the relative importance of phenology, instar susceptibility and instar specific exposure was evaluated. Two insecticides, cypermethrin and dimethoate, were investigated. In the laboratory, topical toxicity to eggs, second-instar larvae and adults was recorded in dose-response experiments. The spatial distribution of larvae and eggs were measured in the field. Deposition of insecticide onto eggs, second-instar larvae and adult specimens was measured at different positions within the crop canopy by use of a dye tracer technique. A temperature-driven population model was constructed to simulate population development of all life stages in the field. The model was based on laboratory measures of growth and development at various temperatures. Mortality due to direct insecticide exposure was calculated as a function of population demography, spatial distribution of individuals, spatial deposition of the insecticide, and stage-specific susceptibility. Cypermethrin had the greatest impact, reducing population size by 19–32%. The life stages most sensitive to cypermethrin were the larval instars. As the population developed from eggs to larvae and imagines, the impact of one spraying first increased and then decreased according to the proportion of larvae in the population. Dimethoate had less effect on the population, i.e. 1·9–7·6% reduction. Dimethoate was most toxic to the egg stage, and consequently the effect on the population decreased as the proportion of eggs decreased due to hatching. The direct effect of insecticide spraying was significantly affected by all three factors investigated, i.e. phenology, life stage susceptibility and stage-specific exposure. The latter factor is composed of both spray flux at various spatial positions in the canopy and the ability of different life stages to retain spray droplets. © 1998 SCI

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1 INTRODUCTION

An important aspect of insecticide effect assessment is the estimation of effects on insect populations in the field for the purpose of protecting the crop against pests or assessing detrimental effects to non-target insects. Information on insecticide toxicity can be obtained from laboratory dose-response assays, but it is difficult to predict effects in the field from laboratory results for a number of reasons. First, the exposure of insects to insecticide spraying is composed of three routes of uptake: direct topical, residual and dietary. The relative importance of these three exposure routes differs among species of different feeding guilds and trophic levels. There is, however, evidence that effects of direct spray

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deposition are of importance for non-target invertebrates exposed to broad-spectrum insecticides like organophosphates and pyrethroids.[1] Secondly, many factors, other than the feeding habits of the species, are known to affect the efficacy of direct insecticide exposure. These factors include: the abiotic environment, the degree of shelter from the crop canopy, the ability of the specimen to retain spray solution, the behaviour of the organism, and the intrinsic toxicity of the insecticide to different developmental stages. Previous studies which have assessed the direct insecticide effects on the basis of actual spray deposition measures in the field have done so on a single developmental stage.[2–4] However, all developmental stages may not be equally exposed to spray deposits. Soil-dwelling stages may pass unexposed and other stages may be in positions of low pesticide deposition. Furthermore, different animals of different life stage have different susceptibilities.[5,6] Therefore assessment of population effects in the field adds valuable information to laboratory data. Field experiments are often difficult to repeat with the same results, due to the above-mentioned fluctuations in abiotic and biotic environment of the studied population. The time of spraying in relation to the phenological stage of the population is one of the factors that may influence the outcome of field effect. This is expected if the population consists of several life stages with different insecticide susceptibilities and with different behaviours, resulting in stage-specific spatial distribution and consequently different exposures.

As it is very laborious to do field studies on the influence of spray timing on population effects of insecticide applications in species with several life stages of differing susceptibilities, other procedures are needed. By the use of mathematical models, it is possible to analyse the importance of spray timing on spray efficacy if data on susceptibility, spatial distribution, insecticide deposition, and developmental rate of the species are available. This may be viewed as steps towards a population toxicological approach, with possibilities of incorporating biotic and abiotic parameters into the assessment of field effects.

The present study aims to study the influence of spray timing in relation to effects of direct topical exposure on populations of the non-target chrysomelid beetle, *Gastrophysa polygoni* L. We wish to do so on the basis of a stage-structured approach, both with respect to actual spray deposition, behaviour in the canopy, and insecticide susceptibility, in order to evaluate the significance of the age-structured approach compared to single-stage assessments. *G. polygoni* is an oligophagous leaf beetle living on the annual weed plants *Fallopia convolvulus* Adans. and *Polygonum aviculare* L. The beetle is a non-target organism in the agro-ecosystem and constitutes a part of the preferred food items for chicks of farmland birds like the grey partridge (*Perdix perdix* L.).[7–8] The species can serve both as a general insect model and as an example of insecticide effects on a non-target population. The authors are aware that quantification of residual and oral effects is necessary before reliable estimates of risk can be made.

2 MATERIALS AND METHODS

In order to estimate the importance of a stage-structured approach compared to single-stage measurements, and to estimate the importance of spray timing on direct topical effects of insecticides, four types of experiments were conducted, i.e. estimates of population composition; spatial distribution of each developmental stage; instar susceptibility to two insecticides; and estimates of pesticide deposition onto different life stages at various positions in the crop canopy. The test species, *G. polygoni*, has three larval instars and the active life stages all live on the aerial parts of the host plants. They are therefore directly exposed to pesticide sprays. The pupal stage is soil dwelling. When imagines emerge they mate and, after a few days, the females start egg-laying. The females oviposit several hundred eggs over a period of several weeks.

2.1 Population composition of *Gastrophysa polygoni* in the field

Absolute measures of population composition often fail because no single sampling technique can describe all instars quantitatively at the same time, and there is no simple way to compare different sampling techniques directly. Therefore population composition of *G. polygoni* at the time of spraying was estimated by use of a population model.

2.1.1 The population dynamical simulation model

The simulation model applied is a stage-distributed physiologically driven population model of the metabolic pool type[9–10] which uses field temperature measurements as the most important variable. The temperature measurements are converted into a physiological time scale (degree-days, i.e. actual temperature minus lower threshold temperature for growth) and any process in the model is defined per degree-day (e.g. survival, reproduction, ageing and growth). The model used two observations from the field, density of adults in spring and first observation of adults, as a starting point. Instar-specific predation rates used in the model were obtained using Sotherton's data.[11]

The dynamics of a population is controlled by the aid of time-invariant distributed delays[12] which is a 'book-keeping device' that keeps track of stage-distribution, performs ageing and incorporates mortality.[13] The dis-

tributed delay adds variation to the average developmental time (in degree-days) of a stage and the variation is described by an Erlang frequency distribution.[9,12] In this model the population dynamics are controlled by two parallel series of distributed delays, two delays for each stage: one to control the numbers and one to control the dry weight. The life stages are egg, three larval instars, prepupae, pupae, non-reproducing adult and adult.

The performance of the simulation model was evaluated by comparing simulation output with field observations. Temperature was measured hourly with a sensor placed 15 cm above ground in the field where sampling was carried out.

In order to estimate the lower threshold temperature (T_0) for growth of *G. polygoni* larvae, which is used in calculations of degree-days, feeding trials were conducted with excised leaves of a host plant. Newly hatched larvae were placed singly on leaves of *F. convolvulus* in a 9-cm Petri dish lined with filter paper. The filter paper was watered with 0·7 ml water and placed in a closed box with a humid paper towel in the bottom, thereby creating approximately 100% RH in the Petri dish. The food plants were grown in a controlled environment chamber at 20°C, 80% RH, and 16 : 8 h light : dark. Larvae were fed at constant ambient temperatures of 10°C, 15°C, 20°C and 25°C, respectively. The relative growth rate was calculated on the basis of fresh weight of the larvae measured after 48 h. Weight was determined at intervals of 24 h until pupation.

2.1.2 Field observations

In order to establish the necessary validation of the above-mentioned simulation model, field sampling was carried out. Sampling of a *G. polygoni* population was conducted within an unsprayed spring barley field at the Kalø Estate in Denmark. First sampling was on 11 May (one week after the first observation of eggs) and the last sampling was on 14 July (one week after peak occurrence of adults of the second generation). At each sampling date, 10 samples were collected along tramlines in the field. Samples were collected by use of a Dietrich vacuum insect suction sampler.[14] One sample comprised five, 12-s suction periods, each covering an area of 0·1 m^2.

2.2 Spatial distribution of *Gastrophysa polygoni* on the host plant

The exposure of insects in the canopy is dependent on their position within that canopy. Therefore, observations of spatial distribution of *G. polygoni* were made in the field. On 5 June the positions of eggs, larvae and adults in the field were determined in an unsprayed spring barley field. The following parameters were recorded for specimens on *F. convolvulus* plants: developmental stage, number and position on the plant. Position categories were top, middle and bottom third of the crop canopy. In each category a sub-division was made (i.e. upper or lower side of the leaves), also observations of individuals on the soil surface were registered. It was not possible to separate L_1 and L_2 in the field, and consequently these two instars were pooled. Because very few adult specimens were present in the field, an earlier laboratory experiment was used[4] as a measure of the expected distribution of adults within the canopy.

2.3 Topical toxicity

Acute toxicities to larval and egg stages were measured at 20°C. Dimethoate 280 g $litre^{-1}$ EC (DLG, Esbjerg Kemi, Denmark) or cypermethrin 100 g $litre^{-1}$ EC (Cyperb, DLG, Denmark) was used in all treatments and diluted with demineralized water which was also used as a control treatment. The pesticide was applied with a manual micro-applicator (Burkard Manufacturing Co. Ltd., Rickmansworth, UK). The applied insecticide doses are given with the data in Table 4. The experimental animals were collected from a base culture. This culture consisted of adult specimens confined in a cage and placed within a controlled environment chamber at 20°C and a light intensity of 359 microeinstein s^{-1} m^{-2}. The light : dark cycle was 16 : 8 h. Whenever the culture reached the third generation it was restarted with specimens collected in the field.

2.3.1 Eggs

Egg batches were collected from the base culture and transferred to an undamaged, excised leaf. Ten eggs were evenly spaced on each leaf. There was only one egg from the same egg-clutch within the same replicate. Three such leaves (replicates) were made for each treatment. The egg-leaves were placed in 9-cm Petri dishes lined with a humid filter paper (0·7 ml water) and subsequently the Petri dishes were placed in a box with a humid towel paper in the bottom. After application of insecticides in 0·23-μl droplets, the numbers of hatched eggs and surviving larvae were determined daily for seven days.

2.3.2 L_2-larvae

Second-instar larvae were collected from the base culture and weighed. Specimens weighing 1–2 mg were chosen for experiments and placed on a leaf in a Petri dish with humid filter paper (0·7 ml water). There were three replicates for each dose and 15 beetles for each

replicate for the dimethoate treatment. In the experiments with cypermethrin, four replicates of 10 specimens were made for each dose. The insecticide was applied in droplets of 0·25 μl. Mortality was determined daily until a stable level (end-point) was reached. Larvae which did not respond to the touch of a soft brush were considered dead.

2.3.3 *Adults*

The toxicity of dimethoate and cypermethrin to adult *G. polygoni* was obtained from the literature.[4,15]

2.3.4 *Statistical analyses*

Probit analysis was carried out to obtain dose-response statistics. Abbott's formula[16] was applied to correct for control mortality and only dead specimens were included in the calculations. The probit analysis was undertaken using weighted regression analysis as recommended by Bliss.[17] Slope and intercept of probit lines were compared by means of a paired *t*-test. The level of significance was 5%, unless otherwise stated.

2.4 Measures of insecticide deposition

A fluorescent tracer was used to estimate pesticide deposition on three different developmental stages of *G. polygoni* placed on the host plant *F. convolvulus*. In the experiments, potted plants were placed within a spring barley field. The crop growth stage was 52–56,[18] which means that one-quarter to one-half of the inflorescence had emerged. The crop density was 891($\pm$88) tillers m^{-2}.

Measures of deposition on adult insects were made on freshly killed specimens, pinned on headless pins and placed on the mid-vein. Only the feet of the beetles were in contact with the leaf surface. Adult beetles placed on the ground were pinned and the pin was positioned on a piece of filter paper. Live eggs and second-instar larvae were placed on the plants shortly before spraying. Eggs were placed on the lower side of the leaves in all three strata (top, middle and bottom third of the crop). Second-instar larvae were placed on both the upper and the lower sides of the leaves in the bottom and middle crop layers. The individuals on the upper side of the leaves were positioned on one series of plants and those on the lower side on another series.

2.4.1 *Spray procedure*

The spraying was carried out with an Azo-sprayer equipped with nine Hardy 110° flat-fan nozzles (type 411016). In all experiments the sprayer was operated with a working pressure of 2 bar. Used in this way the sprayer produced a swath width of 2·2 m and delivered 4·32 litre min^{-1}. Application rates were calibrated to deliver 200 litre ha^{-1} by adjusting walking speed. These conditions are within the operational condition for use of dimethoate and cypermethrin in cereals in Denmark.

In all experiments fluorescein, a fluorescent tracer, was used as a 0·5 g $litre^{-1}$ solution mixed with a normal recommended insecticide dose (i.e. 280 g ha^{-1} for dimethoate and 12·5 g ha^{-1} for cypermethrin. Samples were collected straight after the application and placed in labelled vials with phosphate buffer (pH 6·8, 0·1 M $Na_2HPO_4 + NaH_2PO_4H_2O$), shaken and kept in the dark until analysis. Adults were placed in 2 ml buffer, eggs and larvae in 1 ml buffer. Recovery of known amounts of fluorescein applied to all items was measured. There was agreement between applied and recovered amounts for all items. A sample of the spray formulation was taken for calibration of the volumetric analysis. An aliquot was taken from each vial and the volume of tracer in the solution was determined by fluorescence spectrophotometric analysis (Shimazu RF-530 fluorescent monitor operating at 490 nm excitation and 515 nm emission wavelengths). Blank comparisons were made with buffer solution. A standard calibration curve was made from known amounts of the original spray solution for both 1 and 2 ml buffer. The volume of spray solution in microlitres was converted to microlitres per unit area. The environmental conditions during spraying were 26°C in the shade, a wind speed of 0–2 m s^{-1}, and bright sunshine.

2.4.2 *Surface area determination*

The amounts of pesticide trapped by eggs, second-instar larvae and adults were divided by their surface area, in order to be able to compare the different sample items directly. The areas were determined differently for different life-stages. For adults and second-instar larvae, a standard correlation curve had been established between weight of specimens and the projected area the beetle covered:

Adults: Projected area
$= 7{\cdot}701 \times \text{weight} + 0{\cdot}056,\ n = 35,\ R^2 = 0{\cdot}43$

L_2: Projected area
$= 9770 \times \text{weight} + 0{\cdot}009,\ n = 60,\ R^2 = 0{\cdot}97$

where weight is the fresh weight (g) of the beetle. The projected area was estimated using a digital image processing system. In the present experiments, the specimens were only weighed and from this measure the projected area was calculated from the regression formula above. Surface area conversions were made by correcting for the dorsal surface area of each insect to take account of body curvature. This was done by assuming the adult insect to be ellipsoid, larvae to be cylindrical and eggs to be ellipsoid. Based on these assumptions the actual surface was approximated

by multiplying by factors of 1·5, 1·8 and 1·5 for adults, larvae and eggs, respectively.

2.4.3 Validation of exposure measurements

The deposition measures were validated by placing specimens in a specific site of *F. convolvulus* plants in the canopy and spraying with either water or insecticide in separate trials. Insecticide dosages were the recommended field rates in Denmark for the respective insecticides. The observed mortality was compared to the calculated effects based on the deposition and toxicity data. Spraying conditions and procedures were identical with the deposition measurements described above, except that fluorescein was not applied. These experiments were carried out for eggs and second-instar larvae on the lower side of leaves in the middle third of the crop canopy and for adults applied to the pots. The adults were left for 15 min, so that they could spread over the plant. Immediately after spraying the animals were collected and placed in a Petri dish with uncontaminated host plant foliage. Mortality was registered for all collected specimens for 14 days.

2.4.4 Statistical analyses

A two-way ANOVA for unequal sample sizes (i.e. General Linear Models[19]) was carried out to test for effects of position of targets on spray deposition and between different target items. If significant effects were found, Tukey *t*-tests were performed on mean deposition levels at separate sites. For the validation, water and insecticide were compared for each developmental stage separately by means of a paired-*t*-test.

2.5 Estimates of population effects from direct insecticide deposition

It was assumed that mortality could be computed as a function of the demography, the spatial distribution of the various life stages, deposition of the insecticide at various sites (i.e. spatial distribution of the toxicant), and the toxicity of the insecticide to the different life stages.

A stepwise approach was chosen in order to compare the importance of the factors studied. The first calculation (eqn (1)) assumes that all insecticide is deposited on individuals at a rate equal to the applied field rate (i.e. assuming no drift, dilution, shadowing or volatilization):

$$M = \alpha \times (D \times A) + \beta \tag{1}$$

where M is the predicted mortality, α is the slope of the dose-response line (see Table 4), β is the intercept of the dose-response line, D is the dosage and A is the area of the target. In the second calculation (eqn (2)) the actual deposition measured in a crop canopy for each instar placed at different sites is included:

$$M = \alpha \times Dep + \beta \tag{2}$$

where Dep is the expected deposition on an individual of a specific developmental stage at a specific site. In the third calculation, the spatial distribution of the different developmental stages has been incorporated (eqn (3)):

$$M = \sum_{site} ([\alpha \times Dep + \beta] \times p(site)) \tag{3}$$

where $p(site)$ is the probability of finding the beetle in a specific position. In the fourth calculation the population structure is included (eqn (4)):

$$M = \sum_{\text{site}} \sum_{i=1}^{7} ([\alpha \times Dep + \beta] \times p(site) \times p(i)) \tag{4}$$

where i is the instar (egg, L_1, L_2, L_3, prepupae, pupae and adult) and $p(i)$ is the proportion of the population that are instar 'i'. In the latter calculation it was assumed that prepupae and pupae were not exposed to insecticide, as they are soil-dwelling. Because the actual laboratory measures of deposition and dose-response relationships were made only for one larval instar (L_2) an approximation was made for the effect of the insecticide on L_1 and L_3. For this approximation larvae were assumed to be cylindrical and the relationships between weight and both length and width of the larvae were established in the laboratory. It was then possible to calculate the total surface area of a larva, given its weight. As the entire surface is not subjected to spray deposition the exposed proportion of the surface was estimated by spraying a second-instar larvae and glass strips respectively with a dye in a Potter Spray Tower. The deposition on the larvae and on the glass strip was measured. The relation between these measures was 1·8. By relating the calculated surface area and the projected area, the proportion of the surface available to deposition was estimated to be 0·56. Furthermore, it was assumed that the toxicity of an insecticide was constant on the basis of the dose per body weight for all larval instars. For the calculations, the mean size of an L_1 larva was 0·5 mg and an L_3 was 6·5 mg. Four dates regularly spaced within the normal spraying season were chosen to exemplify the effect of spray timing on insecticide effects on the population (14, 21 and 28 June, 5 July).

3 RESULTS

3.1 Population composition of *Gastrophysa polygoni* in the field

3.1.1 Laboratory experiments

The lower threshold temperature for growth of larvae

was estimated as 6·3°C from the following regression line of growth rates versus temperature:

$$RGR = 0{\cdot}0016(\pm 0{\cdot}0001) \times temp - 0{\cdot}0102(\pm 0{\cdot}0018),$$
$$R^2 = 0{\cdot}89$$

where *RGR* is the relative growth rate and *temp* is the temperature in °C. The duration of each developmental stage in degree days is presented in Table 1.

3.1.2 *Simulation results*

Generally, there is a close correspondence between those instars caught in D-vac samples and the simulated phenology (Fig. 1). The density does not fit precisely, although several of the simulated numbers are within the range of the observed average value ± SEM. The largest deviation between observations and simulation occurs in the mid-July, especially for adults and L_1.

The model prediction of population composition at four selected times of spraying is presented in Table 2.

TABLE 1
Duration of Different Developmental Stages of *Gastrophysa polygoni*

Developmental stage	*n*	*Mean duration (deg.-day)*	*Standard error of mean*
L_1	10	31·5	2·7
L_2	10	40·5	2·0
L_3	10	68·0	2·5
Prepupae	10	53·3	11.3

Duration was measured in degree-days on basis of a lower threshold temperature (T_0) of 6·34°C. The experiments were conducted at 20°C. *n* is the number of replicates.

3.2 Spatial distribution of *Gastrophysa polygoni* on the host plant

In all, 496 eggs, 912 first- and second-instar larvae, 79 third-instar larvae and nine imagines were observed in the field. Eggs were exclusively found on the underside of the leaves and primarily in the lower part of the canopy (81·5%) (Table 3). The relative distribution of first- and second-instar larvae is not different from that of the eggs (χ^2-test) which may indicate that they stay at the site where the eggs were placed. The third-instar larvae are observed higher in the canopy. Adults have a significantly different distribution from any other instar. They are found in high proportions on the stem, which suggests that they are walking all over the plant most of the time. Adults also occur more often on the upper side of the leaves than do the other stages (Table 3).

3.3 Topical toxicity

From the log-transformed dose-response relationships of cypermethrin and dimethoate (Table 4), it is apparent that cypermethrin is, in general, more toxic than dimethoate to eggs and second-instar larvae.

3.4 Measurements of insecticide deposition on insects

Deposition was not significantly different between experiments for the same items placed in the same posi-

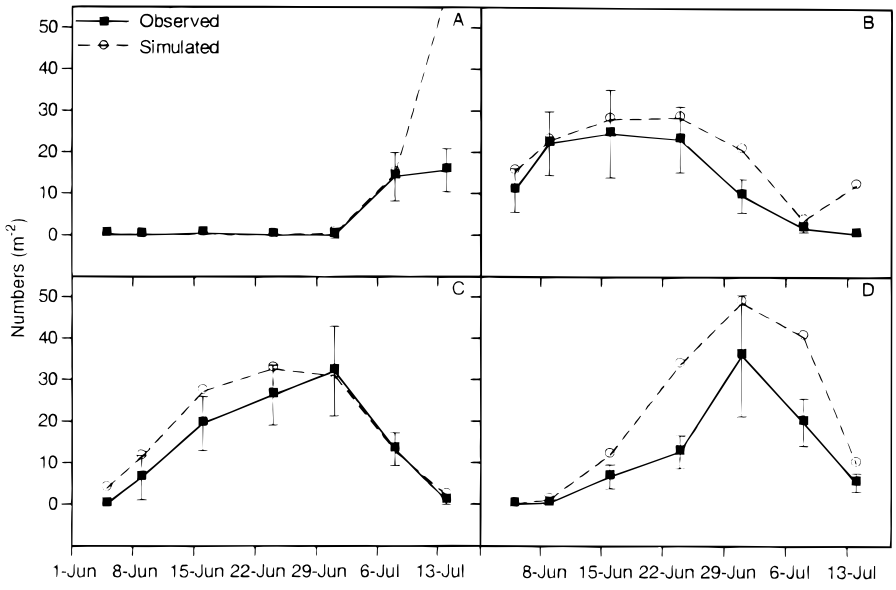

Fig. 1. Comparison of the output of a dynamic population model for *Gastrophysa polygoni* and actual population densities of different life stages caught in the field (A; adults, B; L_1, C; L_2, D; L_3). Bars represent SEM.

TABLE 2
Population Compositions according to Phenological Simulation Model on Four Selected Dates for Spraying

Development stage	*14 June*		*21 June*		*28 June*		*5 July*	
	Density	*Proportion*	*Density*	*Proportion*	*Density*	*Proportion*	*Density*	*Proportion*
Egg	83·6	0·588	71·1	0·439	26·0	0·164	8·9	0·061
L_1	27·5	0·195	28·6	0·177	23·4	0·148	4·3	0·029
L_2	23·5	0·166	31·9	0·197	32·0	0·203	14·1	0·096
L_3	7·1	0·05	28·1	0·173	48·0	0·304	41·8	0·285
Prepupae	0	0	2·2	0·014	23·7	0·150	35·9	0·245
Pupae	0	0	0	0	4·7	0·030	29·0	0·198
Adult	0·2	0·0014	0·1	0·0006	0·1	0·001	11·6	0·087

Density is the estimated beetle density (m^{-2}) in the field, proportion is the proportion of a single life stage in the whole population.

tions (numerous ANOVA tests not presented). Data were therefore pooled. As expected, adult specimens placed on the upper side of the leaves collected most spray solution when in the upper part of the canopy, and deposition decreased down through the canopy, but not significantly (One-way ANOVA, $F = 2{\cdot}37$, $df = 3$, $p = 0{\cdot}0802$; Fig. 2). At soil level, deposition increased compared to the bottom level of the plant. A similar decrease down through the canopy was seen for adults positioned on the stem of the host plant (One-way ANOVA, $F = 2{\cdot}75$, $df = 2$, $p = 0{\cdot}075$); (Fig. 2). The mean values for the stem position were lower than the values for specimens on the upper leaf surface (Tukey *t*-test). Deposition onto larvae on the upper side of the leaves was higher than for all other sample items in the same positions, and contrary to the results for adults there were no differences between the two strata measured (Tukey *t*-test).

Specimens of the beetles positioned on the lower side of the leaves collected less than on the upper side (Tukey *t*-test) (Fig. 2). For eggs, larvae and adult specimens on the lower side of the leaves the deposition rate was, constant down through the canopy (One-way Anova $F = 0{\cdot}19$, $df = 2$ $p = 0{\cdot}828$ for adults, $F = 0{\cdot}39$, $df = 1$, $p = 0{\cdot}54$ for second-instar larvae and $F = 3{\cdot}21$, $df = 2$ $p = 0{\cdot}112$ for eggs). Again larvae collected more spray solution than the other instars (Tukey *t*-test) (Fig. 2).

3.4.1 *Validation of exposure measurements*

There were no significant differences in the survival of specimens treated with water or insecticide in the field experiments for any of the instars sprayed (i.e. eggs, L_2 and adults) (paired *t*-test). This agrees with the calculated (lack of) mortality based on laboratory data.

TABLE 3
Spatial Distribution of Different Instars of *Gastrophysa polygoni* Found on Black Bindweed (*Polygonum convolvulus*) within a Cereal Crop Canopy

Position[a]	*Leaf side*	*Field data*			*Laboratory data*
		Egg	$L_1 + L_2$[b]	L_3	*Adult*
Top	Upper	0	0	0	0·044
	Lower	0	0·034	0·063	0·238
Middle	Upper	0	0·002	0·025	0·008
	Lower	0·186	0·121	0·329	0·076
Bottom	Upper	0	0·011	0·051	0·035
	Lower	0·814	0·822	0·519	0·102
Stem	—	0	0·002	0	0·488
Ground	—	0	0·006	0·013	0·005
n[c]		496	912	79	408

[a] Position categories were top, middle and bottom referring to the position of the beetle in the top, middle or bottom third of the crop canopy.
[b] L_1 and L_2 are pooled because it was not possible to distinguish them in the field.
[c] *n* is the total number of observations for each developmental stage. The laboratory data were obtained from Kjær and Jepson.[4]

TABLE 4
Effects of Dimethoate and Cypermethrin on 168-h Mortality of Egg and 96-h Mortality of Larvae of *Gastrophysa polygoni*

				Replicate					
Instar	*Insecticide*	*Dose*	*n*	*1*	*2*	*3*	*4*	*Mean*	*SEM*
$Y = \alpha \times \log(\text{dose}) + \beta$									
Egg	Dimethoate	0	30	0	0	0	—	0	0
Egg	Dimethoate	0·5	30	0	0·167	0·200	—	0·122	0·062
Egg	Dimethoate	1·0	30	0·167	0·200	0·571	—	0·313	0·129
Egg	Dimethoate	1·5	30	0·375	0·670	0·333	—	0·459	0·110
Probit = 1·849(±0·849) × log(Dose)[a] − 0·467(±0·158), $n = 9$, $R^2 = 0{\cdot}404$									
Egg	Cypermethrin	0	30	0	0·167	0·333	—	0·167	0·096
Egg	Cypermethrin	0·09	30	0·500	0·333	0·285	—	0·373	0·065
Egg	Cypermethrin	0·18	30	0·429	0·400	0·333	—	0·387	0·028
Egg	Cypermethrin	0·36	30	0·500	1·000	0·625	—	0·708	0·150
Egg	Cypermethrin	0·72	30	1·000	0·833	1·000	—	0·944	0·056
Probit = 1·650(±0·371) × (log(Dose) + 1·186(±0·263), $n = 12$, $R^2 = 0{\cdot}664$									
L_2	Dimethoate	0	45	0	0	0	—	0	0
L_2	Dimethoate	50	45	0·533	0·400	0·200	—	0·378	0·097
L_2	Dimethoate	75	45	0·667	0·400	0·200	—	0·422	0·135
L_2	Dimethoate	100	45	0·467	0·467	0·467	—	0·467	0
L_2	Dimethoate	150	45	0·933	0·733	0·667	—	0·777	0·080
Probit = 1·890(±0·775) × log(Dose) − 3·659(±1·499), $n = 12$, $R^2 = 0{\cdot}373$									
L_2	Cypermethrin	0	40	0	0	0·30	0	0·075	0·038
L_2	Cypermethrin	0·069	40	0·30	0·30	0·10	0·50	0·30	0·082
L_2	Cypermethrin	0·14	40	0·30	0·10	0·20	0·30	0·023	0·050
L_2	Cypermethrin	0·328	40	0·90	0·50	0·30	0·60	0·575	0·125
L_2	Cypermethrin	0·654	40	0·60	0·70	0·90	0·60	0·70	0·071
Probit = 1·157(±0·344) × log(Dose) + 0·630(±0·262), $n = 16$, $R^2 = 0{\cdot}448$									
$Y = \alpha \times \log(\text{dose} \times \text{body weight}^{-1}) + \beta$									
Dimethoate, L_2: Probit = 1·890(±0·775) × log(Dose g^{-1}) − 9·142(±3·745), $R^2 = 0{\cdot}373$									
Cypermethrin, L_2: Probit = 1·157(±0·343) × log(Dose g^{-1}) − 2·726(±0·778), $R^2 = 0{\cdot}448$									

[a] Standard errors of regression parameters are presented in parentheses. The mean weight was 0·159(±0·008) mg for eggs and 1·257(±0·315) mg for L_2. Data were fitted to the equation $Y = \alpha \times \log(\text{dose}) + \beta$ or $Y = \alpha \times \log(\text{dose} \times \text{body}) \text{ weight}^{-1}) + \beta$, where Y is the probit value, α is the slope of the regression line and β the intercept. The dose is in ng AI individual^{-1}. Body weight is calculated in g. n is the total number of specimens treated. All experiments were conducted at 20°C.

3.5 Estimates of effects from direct insecticide deposition

For dimethoate the assumption of no loss of insecticide from nozzle to the insect (eqn (1)) predicts a very high mortality for eggs and adults, 0·936 and 0·881 respectively (Table 5), and a moderate mortality for larvae (0·386). When measurements of actual deposition are used (eqn (2)) the estimated mortality drops to a range between 0 and 0·333 depending on position in the canopy. As the eggs occur only on the lower side of the leaves, the implementation of real deposition in the computation reduces egg mortality markedly, which explains the low egg-efficacy calculated by this equation (0·063–0·115). Adults visit all sites, but the effect is low. Mortality estimates for L_2 larvae ranged from 0·020 and up to the level of eqn (1), which is 0·386.

Adding the spatial distribution of the specimens gives no reduction in the estimated mortality for eggs, whereas, for both larvae and adults, it gives an estimate close to the minimum effect in eqn (2).

The relative importance of the difference factors is somewhat different for cypermethrin. In eqn (1) cypermethrin has a high estimated effect on the egg stage and L_2 larvae (0·767 and 0·871, respectively), whereas it is practically non-toxic to adults at the rates applied. Using actual measures of deposition reduces the effect on eggs, as for dimethoate. For adults no reduction is relevant, as it was already non-toxic in the 'no-loss scenario', and, for larvae, the reduction depends on position in the canopy. Consequently, the distribution of the specimens is important for the larval instars and not for the other stages.

Equation (4) is used to calculate the effect of spraying at four different phenological dates. The response to spraying date is different for the two insecticides. The effect of dimethoate spraying decreased with time from 0·076 to 0·019, and was always lower than the effect of

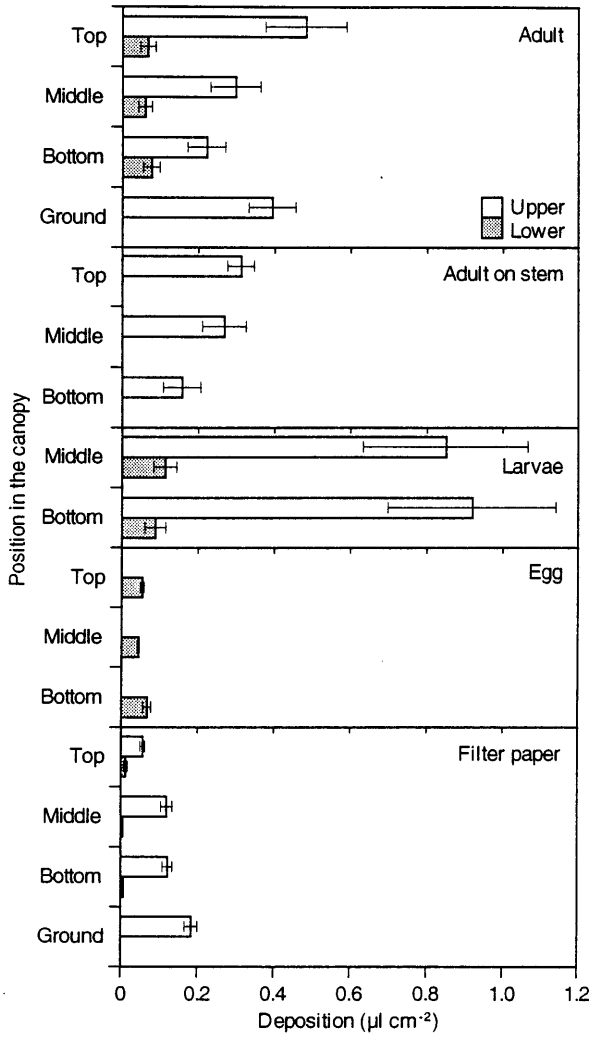

Fig. 2. Spray deposition on different developmental stages of *Gastrophysa polygoni* placed on either upper or lower side of leaves in the top, middle or bottom third of the crop canopy, or placed on the ground. Bars represent SEM.

cypermethrin. For cypermethrin, the effect increased from 0·241 to 0·328 when spray timing changed from first to third time point, followed by a sharp decrease down to 0·194 on the fourth and last date.

4 DISCUSSION

The results suggest that the direct insecticidal effect is governed by a complex interaction between a range of factors. The life stage most sensitive to insecticides differs for the two compounds studied; the relative exposure rate is stage-specific, and the timing of the spraying will determine the age structure of the population present. The results are in agreement with the conclusions of Stark *et al.*[20] and Stark & Wennegren[21] that a stage-structured approach including measurements of relative exposure rates, and behaviour of the animals, would improve predictions of field effects from laboratory studies. This results is also in keeping with the general increase in the use of stage structuring in population dynamics.[22–26]

An alternative to a stage-structured approach in ecotoxicology is the use of the 'most sensitive stage' as a predictor of population effects. In the case of *G. polygoni*, this may be appropriate for a relatively persistent compound (present in lethal concentrations for a whole life cycle), in which the most sensitive stages cannot escape exposure. For short-lived compounds, such as the insecticides studied, this approach over-estimates mortality if the population consists of a mixture of life stages. An effect prediction based on the most sensitive stage would for *G. polygoni*, give a maximum mortality of 0·105 (higher by a factor of 1·3) for dimethoate applied at the recommended field rate, and 0·522 (a factor of 1·6 higher) for cypermethrin.

The estimated population effect of dimethoate decreased over time, whereas cypermethrin had an increasing effect until the last date, where a large decrease occurred. These differences arise because *G. polygoni* is most affected by dimethoate in the egg stage, while cypermethrin is more toxic to the larvae. At the first simulated spraying date, a relatively large proportion of the population is in the egg stage, but, as time goes by, the number of eggs is reduced and the effect of dimethoate consequently declines. The proportion of larvae increases over the first three simulated spraying dates (Table 2), after which many specimens enter prepupae and pupal stages, which are assumed to be practically unexposed. This explains the increasing effect of cypermethrin over the three first dates followed by a decrease on the fourth and latest simulated spraying date.

The calculations made here emphasise that the time of spraying affects the effect of the insecticides for populations occuring in separate cohorts. The importance of the timing of the pesticide application was also stressed by Waage *et al.*[27] and Brown[28] who based their results on deterministic models, taking only the application time in relation to a hypothetical density- dependent factor into consideration. They did not include stage structuring in their models. The results from the modelling presented in this paper are operative in a real world system by generating a stage distribution at any time of the growth season. This makes it possible to include differential behaviour, ability to retain spray solution and susceptibility of the different life stages in consideration in the estimation of the population effect of a pesticide application. Although the use of stage-distributed models is increasing, they still have not been implemented much in the estimation of pesticide effects. The calculations of population effects over time were made under the assumption that deposition patterns do not change between the selected times of spraying. This assumption is not valid according to the data of Cilgi & Jepson[2] and Jepson.[29] Jepson *et al.*[1] found the lowest deposition in the bottom of a barley field around growth stage 50

TABLE 5
Comparisons of Calculated Effect of Insecticide Spraying to *Gastrophysa polygoni* at Recommended Field Rate which Including Different Factors

Calculation no. / Instar	Date	*Egg*		L_2		*Adult*		*Population*	
		Dim[a]	*Cyp*[a]	*Dim*	*Cyp*	*Dim*	*Cyp*	*Dim*	*Cyp*
1		0.936	0·767	0·386	0·871	0·881	0·0008	nr[b]	nr
2 min.		0·063	0·023	0·020	0·519	≈0	≈0	nr	nr
max.		0·115	0·045	0·333	0·850	0·09	≈0	nr	nr
3		0·105	0·041	0·021	0·522	0·006	≈0	nr	nr
4	14 June	nr	nr	nr	nr	nr	nr	0·076	0·241
	21 June	nr	nr	nr	nr	nr	nr	0·064	0·295
	28 June	nr	nr	nr	nr	nr	nr	0·032	0·328
	5 July	nr	nr	nr	nr	nr	nr	0·019	0·194

[a] Dim: dimethoate; Cyp: cypermethrin.
[b] The proposed calculation is not relevant (nr). The first calculation assumes that all insecticide is deposit on encountered items at a rate equal to the recommended field rate; in the second calculation the actual deposition measured in a crop canopy is included. In the third calculation also spatial distribution of the different development stages has been incorporated. Finally in calculation 4 the population composition is added. This last calculation was made for four population compositions (i.e. four different spraying dates) with the assumption that the deposition did not change over this time-span of four weeks.

due to shadowing by the crop. In the present study the growth stage was 52–56, and therefore data represent a 'lowest effect scenario' with respect to insecticide deposition and thus estimate a minimum population effect. This is supported by data of Kjær & Jepson,[4] who in a similar calculation found that approximately 40% of the adults would die from a recommended field rate of dimethoate sprayed in a crop at growth stage 92. However, crop densities vary between studies, so accurate comparisons are not possible.

Since the calculations are based on a number of estimated factors, leaving no opportunity for statistical verification, it is necessary to discuss the reliability of the single factor in the calculations. The population composition was estimated by use of a dynamic population simulation model. This model was validated against actual population measurements made in the field. The largest deviation was found in mid-July when the population consisted mainly of adults. This deviation may arise because the model assumes unlimited food resources. In reality, the two host plants, *F. convolvulus* and *P. aviculare*, had almost totally disappeared from the field in early July due to severe drought. In the field, a food shortage causes newly emerged *G. polygoni* adults to enter diapause in the soil.[30] This is probably the reason why a second generation of larvae was not observed in the field. However, the data used for estimates of population effects are based on data from the period before the food shortage, when the simulation of the population demography fitted field observations reasonably well.

The pattern of deposition down through the crop canopy is comparable to other data presented for both adult beetles[2–4] and glass models of larvae,[31] but the absolute level of deposition is different. The difference may occur because of the developmental stage of the crop and different crop density (there was a two-fold difference in tiller density between the studies and two cereal crop species were involved).

The measurement of spatial distribution of different developmental stages in the canopy was made with a high number of observations, which make the data reliable for the existing environmental conditions (i.e. dry weather with low winds and no rain and a high temperature (i.e. 26°C in the shade). Under other environmental conditions the distribution might have been different.

The topical dose-response relationships revealed a higher toxicity of cypermethrin compared to dimethoate. For other herbivorous insects a similar relationship has been observed between different pyrethroids and dimethoate.[32–34] The exact relationship is difficult to assess because it changes in relation to temperature and other abiotic factors, but the literature gives no reason to mistrust the relative toxicities measured in the present paper.

5 CONCLUSION

The present data set revealed that all factors studied, i.e. population composition, spatial distribution of single developmental stages, instar susceptibility to the investigated insecticides and pesticide deposition within the crop canopy and onto different life stages are important to the estimated effects of insecticide spraying. The importance of the factors also depends on the pesticide

in use. The most sensitive life-stage differed between the two insecticides investigated, which resulted in a different response to changes in time of spraying. Data indicate that the present approach to risk assessment is more accurate than the commonly used single-instar procedures. The applied procedure is much enhanced by the use of a stage-structured population model.

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REFERENCES

1. Jepson, P. C., Efe, E. & Wiles, J. A., The toxicity of dimethoate to predatory Coleoptera: developing an approach to risk analysis for broad-spectrum pesticides. *Arch. Environ. Contam. Toxicol.*, (1995) 500–7.
2. Cilgi, T. & Jepson, P. C., The use of tracers to estimate the exposure of beneficial insects to direct pesticide spraying in cereals, *Ann. Appl. Biol.*, **121** (1992) 239–47.
3. Thacker, J. R. M. & Hickman, J. M., Techniques for investigating the routes of exposure of carabid beetles to pesticides. In: *The role of gound beetles in ecological and environmental studies*, ed. N. E. Stark. Intercept. Andover, UK, 1990, pp. 105–13.
4. Kjær, C. & Jepson, P. C., The toxic effects of direct pesticide exposure for a non-target weed-dwelling chrysomelid beetle (*Gastrophysa polygoni*) in cereals. *Environ. Toxicol. Chem.*, **14** (1995) 993–9.
5. Busvine, J. R., *A critical review of the techniques for testing insecticides.* Commonwealth Agriculture Bureaux, Slough, 1971.
6. Croft, B. A., *Arthropod biological control agents and pesticides.* John Wiley & Sons, New York, 1989.
7. Potts, G. R. *The Partridge: pesticides, predation and conservation* Collins, London 1986.
8. Sotherton, N. W. & Moreby, S. J., The importance of beneficial anthropods other than natural enemies in cereal fields. *Asp. Appl. Biol.*, **31** (1992) 11–18.
9. Gutierrez, A. P., Baumgärtner, J. U. & Summers, C. G., Multitrophic models of predator-prey energetics. *Can Ent.*, **116** (1984) 923–63.
10. Graf, B., Baumgärtner, J. & Gutierrez, A. P., Modeling agroecosystem dynamics with the metabolic pool approach. *Mitteilungen der Schweizerischen Entomoligischen Gesellschaft*, **63** (1990) 465–76.
11. Sotherton, N. W., Predation of a chrysomelid beetle (*Gastrophysa polygoni*) in cereals by polyphagous predators. *Ann. Appl. Biol.*, **101** (1982) 196–9.
12. Manetch, T. J., Time-varying distributed delays and their use in aggregate models of large systems. *IEEE Transactions on Systems. Man and Cybernetics*, **6** (1976) 547–52.
13. Vansickle, J., Attrition in distributed delay models. *IEEE Transactions on Systems, Man and Cybernetics*, **7** (1977) 635–8.
14. Dietrick, E. J., An improved backpack motorised fan for suction sampling of insect populations. *J. Econ. Entomol.*, **54** (1961) 394–5.
15. Nørregaard, M., Residualoptagelse og effekt af pyrethroidet cypermethrin på Pileurtsbladbillen (*Gastrophysa polygoni* L.) (Coleoptera: Chrysomelidae). *MSc thesis*, University of Aarhus, Århus, Denmark, 1996 (in Danish).
16. Abbott, W. S., A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18** (1925) 265–7.
17. Bliss, C. I., The calculation of the dosage-mortality curve. *Ann. Appl. Biol.*, **22** (1935) 134–67.
18. Zadoks, J. C., Chang, T. T. & Konzak, C. F., A decimal code for the growth of cereals. *Weed Res.*, **14** (1974) 415–21.
19. SAS Institute Inc. *SAS/STAT User's guide.* Release 6.03 Edition, Cary, 1988.
20. Stark, J. D., Jepson, P. C. & Mayer, D. F., Limitations to use of topical toxicity data for predictions of pesticide side effects in the field. *J. Econ. Entomol.*, **88** (1995) 1081–8.
21. Stark, J. D. & Wennergren, U., Can population effects of pesticides be predicted from demographic toxicological studies? *J. Econ. Entomol.*, **88** (1995) 1089–96.
22. Gurney, W. S. C., Nisbet, R. M. & Lawton, J. H., The systematic formulation of tractable single-species population models incorporating age structure. *J. Anim. Ecol.*, **52** (1983) 479–95.
23. Bellows, T. S. & Hassel, M. P., The dynamics of age-structured host–parasitoid interactions. *J. Anim. Ecol.*, **57** (1988) 259–68.
24. Diekkrüger, B. & Röske, H., Modelling the population dynamics of *Isotoma notabilis* (Collembola) on sites of different agricultural usage. *Pedobiologia*, **39** (1995) 58–73.
25. Gutierrez, A. P., *Applied population biology. A supply–demand approach.* John Wiley & Sons, New York, 1996, 300 pp.
26. Munns, W. R., Black, D. E., Gleason, T. R., Salomon, K., Bengtson, B. & Gutjahr-Gobell, R., Evaluation of the effects of dioxin and PCBs on *Fundulus heteroclitus* populations using a modeling approach. *Environ. Toxicol. Chem.*, **16** (1977) 1074–81.
27. Waage, J. K., Hassel, M. P. & Godfray, H. C. J., The dynamics of pest–parasitoid–insecticide interactions. *J. Appl. Ecol.*, **22** (1985) 825–38.
28. Brown, R. A., Pesticides and non-target terrestrial invertebrates: An industrial approach. In: *Pesticides and non-target invertebrates*, ed. P. C. Jepson, Intercept Limited, Wimborne, Dorset, 1989, pp. 19–42.
29. Jepson, P. C., Ecological insights into risk analysis: the side-effect of pesticides as a case study. *Sci. Total Environ., Suppl. 2*, (1993) 1547–66.
30. Hilterhaus, V., Biologish-ökologische Untersuchungen an Blattkäfern der Gattungen *Lema* und *Gastroidea* (Chrysomelidae, Col.). (Ein Beitrag zur Agrarökologie). *Z. Angew. Zool.*, **17** (1965) 257–95.
31. Follett, P. A., Gould, F., Leidy, R. & Bradley, J. R., Deposition of insecticide on glass models of lepidopterous larvae. *J. Econ. Entomol.*, **87** (1994) 1400–6.
32. El-Ghareeb, A. M. & Mannaa, S. H., Impact of mortality determination period and post-treatment temperature on toxicity of synthetic pyrethoids and anticholinesterases to *Spodoptera littoralis* (Boisd.). *Assiut J. Agric. Sci.*, **23** (1992) 311–25.
33. Sinha, S. N., Lakhani, K. H. & Davis, B. N. K., Studies on the toxicity of insecticidal drift to the first instar larvae of the Large White Butterfly *Pieris brassicae* (Lepidoptera: Pieridae). *Ann. Appl. Biol.*, **116** (1990) 27–41.
34. Turner, D. E., The effect of post-treatment temperature on the toxicity of lambda-cyhalothrin and dimethoate to the aphids *Rhopalosiphum padi* and *Sitobion avenae.* Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, Germany, 1993, unpublished report.